(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 February 2004 (12.02.2004)

PCT

(10) International Publication Number
WO 2004/013329 A1

(51) International Patent Classification[7]: C12N 15/10, C12M 1/33, G01N 30/00, 27/447, B01J 19/00, C12M 1/42

(21) International Application Number: PCT/GB2003/003328

(22) International Filing Date: 1 August 2003 (01.08.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0218009.9 2 August 2002 (02.08.2002) GB

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO,

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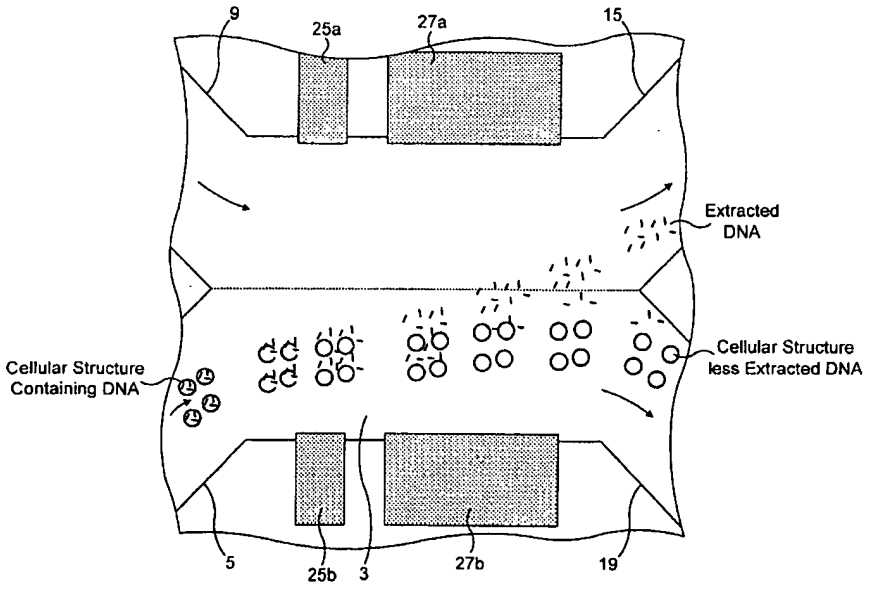
(54) Title: DNA EXTRACTION MICROCHIP, SYSTEM AND METHOD



(57) Abstract: A DNA extraction microchip, system for and method of extracting DNA from a sample including DNA-containing material, including: a main channel through which first and second parallel flows are in use delivered, the first flow being of a sample including DNA-containing material and the second flow being of a separation medium; and an electrode unit for applying a first, electroporation field to the first flow of sample to including DNA-containing material, the electroporation field being such as to effect electroporation of DNA-containing material, and a second, electroseparation field across the main channel, the electroseparation field being such as to effect electroseparation of DNA from the electroporated DNA-containing material in the first flow into the second, parallel flow.

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SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

DNA EXTRACTION MICROCHIP, SYSTEM AND METHOD

The present invention relates to a DNA extraction microchip, system for and method of extracting DNA from a sample including DNA-containing material, typically for the extraction and separation of plasmid DNA from bacterial cells or cell debris, and other more complex DNA sample media, such as white blood cells, saliva, and even naked nuclei for genomic DNA. In particular, the present invention relates to a system for and method of extracting DNA from a sample including DNA-containing material where utilizing a microfabricated DNA extraction device.

Many of the techniques used in genetic analysis have been implemented in microfabricated devices, thereby decreasing analysis times considerably. These techniques include those for the amplification, separation and sequencing of DNA fragments.

The rate-determining step for genetic analysis is often, however, the step of extracting DNA from DNA-containing material, such as bacterial cells or cell debris, as this step of extraction is particularly time consuming. As yet, no microfabricated device has been developed for the extraction of DNA from DNA-containing material.

It is thus an aim of the present invention to provide a microfabricated DNA extraction device for extracting DNA from a sample including DNA-containing material, and a related DNA extraction system and method.

In one aspect the present invention provides a DNA extraction microchip for extracting DNA from a sample including DNA-containing material, the microchip including: a main channel through which first and second parallel flows are in use delivered, the first flow being of a sample including DNA-containing material and the second flow being of a separation medium; and an electrode unit for applying a first, electroporation field to the first flow of sample including DNA-containing material, the electroporation field being such as to effect electroporation of DNA-containing material, and a second, electroseparation field across the main channel, the electroseparation field being such as

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to effect electroseparation of DNA from the electroporated DNA-containing material in the first flow into the second, parallel flow.

Preferably, the main channel has a length of from about 1.5 mm to about 12 mm.

Preferably, the main channel has a width of from about 100 μm to about 1.04 mm.

More preferably, the main channel has a width of from about 100 μm to about 240 μm.

Preferably, the main channel has a depth of from about 10 μm to about 100 μm.

Preferably, the microchip further includes: a sample delivery channel fluidly connected to one, upstream end of the main channel through which the first flow of sample including DNA-containing material is in use delivered; and a separation medium delivery channel fluidly connected to the one end of the main channel through which the second flow of separation medium is in use delivered.

More preferably, the sample delivery channel is connected to one side of the one end of the main channel such that the first flow is along the one side of the main channel, and the separation medium delivery channel is connected to the other side of the one end of the main channel such that the second flow is along the other side of the main channel.

Preferably, the sample delivery channel is inclined at an acute angle to the first and second flows.

Preferably, the separation medium delivery channel is inclined at an acute angle to the first and second flows.

More preferably, the acute angle is approximately 45 degrees.

Preferably, the microchip further includes: a DNA outlet channel fluidly connected to the other, downstream end of the main channel through which the second flow of separation medium entraining extracted DNA is in use directed.

More preferably, the DNA outlet channel is connected to the other side of the other end of the main channel such that the second flow is directed therethrough.

Preferably, the DNA outlet channel is inclined at an acute angle to the first and second flows.

More preferably, the acute angle is approximately 45 degrees.

Preferably, the microchip further includes: a sample waste channel fluidly connected to the other end of the main channel through which the first flow of sample, less extracted DNA, is directed.

More preferably, the sample waste channel is connected to the one side of the other end of the main channel such that the first flow is directed therethrough.

Preferably, the sample waste channel is inclined at an acute angle to the first and second flows.

More preferably, the acute angle is approximately 45 degrees.

In one embodiment the electrode unit comprises first, electroporation electrodes and second, electroseparation electrodes disposed downstream of the electroporation electrodes.

Preferably, the electroseparation electrodes are disposed adjacent the main channel.

In one embodiment the electroporation electrodes are disposed adjacent the main channel.

In another embodiment the electroporation electrodes are disposed adjacent the sample delivery channel.

Preferably, the electroseparation electrodes each have a length of from about 750 μm to about 10 mm.

Preferably, the electroporation electrodes each have a length of from about 150 μm to about 1 mm.

In another embodiment the electrode unit comprises combined electroporation and electroseparation electrodes disposed adjacent the main channel.

In another aspect the present invention provides a DNA extraction system for extracting DNA from a sample including DNA-containing material, the system comprising: the above-described microchip.

Preferably, the system further comprises: a sample delivery unit for delivering a flow of a sample including a DNA-containing material to the microchip.

Preferably, the system further comprises: a separation medium delivery unit for delivering a flow of a separation medium to the microchip.

In one embodiment the sample delivery unit and the separation medium delivery unit are operable to deliver flows having substantially the same flow rate.

In another embodiment the sample delivery unit and the separation medium delivery unit are operable such that a flow rate of the delivered separation medium is higher than a flow rate of the delivered sample.

Preferably, the system further comprises: a pulse generator for applying electroporation voltage pulses across the electroporation electrodes.

Preferably, the pulse generator is configured to generate electroporation voltage pulses at a voltage corresponding to an electroporation field strength of from about 500 V/cm to about 12.5 kV/cm.

Preferably, the electroporation voltage pulses have a duration of from about 20 μs to about 1 ms.

Preferably, the electroporation voltage pulses have an interval of from about 2 ms to about 100 ms.

Preferably, the system further comprises: a voltage supply for applying an electroseparation voltage across the electroseparation electrodes.

Preferably, the voltage supply is configured to generate an electroseparation voltage at a voltage corresponding to an electroseparation field strength of from about 60 V/cm to about 700 V/cm.

In a further aspect the present invention provides a DNA extraction method for extracting DNA from a sample including DNA-containing material, the method comprising the steps of: providing a DNA extraction microchip including a main channel; delivering first and second parallel flows through the main channel, the first flow being of a sample including DNA-containing material and the second flow being of a separation medium; applying an electroporation field to the first flow of sample including DNA-containing material such as to effect electroporation of DNA-containing material; and applying an electroseparation field across the main channel such as to effect electroseparation of DNA from the electroporated DNA-containing material in the first flow into the second, parallel flow.

Preferably, the first flow is along one side of the main channel, and the second flow is along the other side of the main channel.

Preferably, the microchip includes a DNA outlet channel fluidly connected to one, downstream end of the main channel, and further comprising the step of: directing the second flow of separation medium entraining extracted DNA through the DNA outlet channel.

More preferably, the DNA outlet channel is connected to the other side of the one end of the main channel such that the second flow is directed therethrough.

In one embodiment the electroseparation field is applied across electroseparation electrodes disposed adjacent the main channel.

In one embodiment the electroporation field is applied across electroporation electrodes disposed adjacent the main channel.

In another embodiment the electroporation field is applied across electroporation electrodes upstream of the main channel.

In another embodiment the electroporation field and the electroseparation field are applied across combined electroporation and electroseparation electrodes disposed adjacent the main channel.

Preferably, the electroporation field comprises a pulsed field of electroporation voltage pulses.

Preferably, the electroporation voltage pulses are at a voltage corresponding to an electroporation field strength of from about 500 V/cm to about 12.5 kV/cm.

Preferably, the electroporation voltage pulses have a duration of from about 20 μs to about 1 ms.

Preferably, the electroporation voltage pulses have an interval of from about 2 ms to about 100 ms.

Preferably, the electroseparation field comprises a substantially continuous field of an electroseparation voltage across the electroseparation electrodes.

Preferably, the electroseparation voltage is at a voltage corresponding to an electroseparation field strength of from about 60 V/cm to about 700 V/cm.

In one embodiment the first and second flows have substantially the same flow rates.

In another embodiment a flow rate of the second flow is higher than a flow rate of the first flow.

In providing for DNA extraction, the present invention utilizes aspects of electroporation and electroseparation, in particular electroseparation as employed in electrical split flow-thin (E-SPLITT) cell flow fractionation.

Electroporation is a process in which the permeability of a cellular structure is transiently increased by the application of a voltage pulse thereto. This process is commonly used to introduce foreign DNA or chromosomes into cells. In the present invention, however, the contrary effect of electroporation is utilized, that is, the ability to enable the release of DNA from cellular structures.

Electroseparation is a process in which charged components are electrophoretically separated in an electric field. DNA, in being negatively-charged, can be separated from components which have a positive charge or no charge, thereby enabling the separation of DNA from the host cellular structure. As applied to E-SPLITT fractionation, which provides two juxtaposed, parallel flows, that is, a sample flow and a separation medium flow, electroseparation is used to separate charged sample components from the sample flow to the separation flow.

Preferred embodiments of the present invention will now be described hereinbelow by way of example only with reference to the accompanying drawings, in which:

Figure 1 schematically illustrates a DNA extraction system in accordance with a first embodiment of the present invention;

Figure 2 schematically represents the electroporation of DNA-containing material and the subsequent electroseparation of DNA from the electroporated DNA-containing material in the operation of the DNA extraction system of Figure 1;

Figure 3 schematically illustrates a DNA extraction system in accordance with a second embodiment of the present invention; and

Figure 4 schematically illustrates a DNA extraction system in accordance with a third embodiment of the present invention.

Figures 1 and 2 illustrate a DNA extraction system in accordance with a first embodiment of the present invention.

The DNA extraction system comprises a microfabricated DNA extraction device 1, in this embodiment fabricated as a substrate chip, into which a sample including DNA-containing material is introduced and from which DNA is extracted.

The DNA extraction device 1 includes a main channel 3 through which a first flow of a sample including DNA-containing material, typically bacterial cells or cell debris, is driven, and the DNA-containing material is first electroporated to release contained DNA and subsequently separated into a second, parallel flow of a separation medium, as will be described in more detail hereinbelow.

In this embodiment the main channel 3 has a length of 2.5 mm, a width of 140 μm and a depth of 20 μm. In preferred embodiments the main channel 3 has a length of from about 1.5 mm to about 12 mm, a width of from about 100 μm to about 1.04 mm, preferably about 100 μm to about 240 μm, and a depth of from about 10 μm to about 100 μm.

The DNA extraction device 1 further includes a sample delivery channel 5 which is fluidly connected to one, upstream end of the main channel 3 through which a first flow of a sample including DNA-containing material is delivered to the main channel 3. The sample delivery channel 5 includes a sample inlet port 7 into which a flow of a sample including DNA-containing material is delivered to the sample delivery channel 5.

In this embodiment the sample delivery channel 5 is fluidly connected to one side of the upstream end of the main channel 3 such that the flow of sample is along one side, here one half, of the main channel 3. In this embodiment the sample delivery channel 5 is inclined at an acute angle, here about 45 degrees, to the flow direction through the main channel 3.

The DNA extraction device 1 further includes a separation medium delivery channel 9 which is fluidly connected to the upstream end of the main channel 3 through which a flow of a separation medium, in this embodiment a buffer solution, is delivered to the main channel 3. The separation medium delivery channel 9 includes a separation medium inlet port 11 into which a flow of separation medium is delivered to the separation medium delivery channel 9.

In this embodiment the separation medium delivery channel 9 is fluidly connected to the other side, here the other half, of the upstream end of the main channel 3 such that the flow of separation medium is along the other side of the main channel 3 in parallel relation to the flow of sample through the main channel 3. In this embodiment the separation medium delivery channel 9 is inclined at an acute angle, here about 45 degrees, to the flow direction through the main channel 3.

The DNA extraction device 1 further includes a DNA outlet channel 15 which is fluidly connected to the other, downstream end of the main channel 3 at the other side, in this embodiment the other half, of the main channel 3, that is, the same side of the main channel 3 as to which the separation medium inlet channel 9 is connected, through which the flow of separation medium entraining extracted DNA is directed. The DNA outlet channel 15 includes a DNA outlet port 17 through which the flow of separation medium

entraining extracted DNA is delivered, typically collected for subsequent analysis or connected in-line to downstream equipment.

In this embodiment the separated DNA outlet channel 15 is inclined at an acute angle, here about 45 degrees, to the flow direction through the main channel 3.

The DNA extraction device 1 further includes a sample waste channel 19 which is fluidly connected to the downstream end of the main channel 3 at the one side, in this embodiment the one half, of the main channel 3, that is, the same side of the main channel 3 as to which the sample delivery channel 5 is connected, through which the flow of sample, less the extracted DNA, is directed. The sample waste channel 19 includes a sample waste outlet port 21 through which the flow of sample, less the extracted DNA, is delivered.

In this embodiment the sample waste channel 19 is inclined at an acute angle, here about 45 degrees, to the flow direction through the main channel 3.

In this embodiment the sample delivery channel 5, the separation medium delivery channel 9, the DNA outlet channel 15 and the sample waste channel 19 each have a length of 5 mm, a width of 70 μm and a depth of 20 μm. In preferred embodiments the sample delivery channel 5, the separation medium delivery channel 9, the DNA outlet channel 15 and the sample waste channel 19 each have a length of from about 2 mm to about 10 mm, a width of from about 50 μm to about 520 μm, preferably about 50 μm to about 120 μm, and a depth of from about 10 μm to about 100 μm.

The DNA extraction device 1 further includes an electrode unit 23 for applying electroporation and electroseparation fields across the width of the main channel 3, such as to effect electroporation of DNA-containing material delivered therethrough and electroseparation of DNA from the electroporated DNA-containing material.

As described hereinabove, electroporation is a process whereby the permeability of a cellular structure, such as cells or cell debris, is transiently increased, which increased permeability enables the release of DNA from cellular structures.

As also described hereinabove, electroseparation is the electrophoretic separation of charged components in an electric field. DNA, in being negatively-charged, can be separated from components which have a positive charge or no charge, thereby enabling the separation of DNA from the host cellular structure. Moreover, as the speed of electrophoretic migration is determined by the mass-to-charge ratio, and DNA is uniformly negatively charged, and hence has a uniform mass-to-charge ratio, DNA fragmentation can be accommodated.

In this embodiment the electrode unit 23 comprises a first pair of electroporation electrodes 25a, 25b, and a second pair of electroseparation electrodes 27a, 27b which are disposed downstream of the first pair of electroporation electrodes 25a, 25b, with the electrodes 25a, 25b, 27a, 27b of each of the pairs being disposed on opposed sides of the main channel 3.

In this embodiment the electrodes 25a, 25b, 27a, 27b are planar elements, here formed of chromium, which are located at the lower surface of the main channel 3. In another embodiment the electrodes 25a, 25b, 27a, 27b could be formed over the respective elongate sides of the main channel 3. In other embodiments the electrodes 25a, 25b, 27a, 27b could be formed of gold or platinum.

The first pair of electroporation electrodes 25a, 25b, which are disposed upstream of the second pair of electroseparation electrodes 27a, 27b, are effective to cause the electroporation of DNA-containing material on the application of an electroporation voltage thereacross.

In this embodiment the electroporation electrodes 25a, 25b are spaced 200 μm from the upstream end of the main channel 3, and have a length of 300 μm, a width of 100 μm and extend 20 μm into the main channel 3 (corresponding to an electrode spacing of 100 μm).

In preferred embodiments the electroporation electrodes 25a, 25b have a length of from about 150 μm to about 1 mm. In a preferred embodiment the electroporation electrodes 25a, 25b are coated with a barrier material, such as SiO_2, to inhibit degradation.

The second pair of electroseparation electrodes 27a, 27b, which are disposed downstream of the first pair of electroporation electrodes 25a, 25b, are effective to cause the electroseparation of DNA from the electroporated DNA-containing material on the application of an electroseparation voltage thereacross.

In this embodiment the electroseparation electrodes 27a, 27b are spaced 300 μm from the electroporation electrodes 25a, 25b, and have a length of 1.5 mm, a width of 100 μm and extend 20 μm into the main channel 3 (corresponding to an electrode spacing of 100 μm). In preferred embodiments the electroseparation electrodes 27a, 27b have a length of from about 750 μm to about 10 mm. In a preferred embodiment the electroseparation electrodes 27a, 27b are coated with a barrier material, such as SiO_2, to inhibit degradation.

In this embodiment the DNA extraction device 1 is fabricated from two stacked planar substrate plates, here a lower plate composed of microsheet glass, and an upper plate composed of poly (dimethylsiloxane) (PDMS). The electrode pattern was put down on the lower glass plate using a direct-write laser system to expose a positive photoresist on a chromium coating and subsequent etching. The PDMS plate, which includes wells defining the main channel 3, the sample delivery channel 5, the separation medium delivery channel 9, the DNA outlet channel 15 and the sample waste channel 19, was formed using a glass master, which master was formed using a direct-write laser system to expose a positive photoresist and subsequent etching of the glass plate.

The DNA extraction system further comprises a sample delivery unit 29 which is operable to deliver a predeterminable flow rate of a sample including a DNA-containing material to the sample inlet port 7 of the sample delivery channel 5. In this embodiment the sample delivery unit 29 comprises a pump, here a syringe pump.

In this embodiment the sample delivery unit 29 is operated to deliver a flow of sample at a flow rate of 16.7 mm/s. In preferred embodiments the sample delivery unit 29 is operated to deliver a flow of sample at a flow rate of from about 5.6 mm/s to about 140 mm/s.

The DNA extraction system further comprises a separation medium delivery unit 31 which is operable to deliver a predeterminable flow rate of separation medium to the separation medium inlet port 11 of the separation medium delivery channel 9. In this embodiment the separation medium delivery unit 31 comprises a pump, here a syringe pump.

In this embodiment the separation medium delivery unit 31 is operated to deliver a flow of separation medium at a flow rate of 16.7 mm/s. In preferred embodiments the separation medium delivery unit 31 is operated to deliver a flow of separation medium at a flow rate of from about 5.6 mm/s to about 140 mm/s.

The DNA extraction system further comprises a pulse generator 33 which is connected to the electroporation electrodes 25a, 25b and operable to apply electroporation voltage pulses thereacross, which voltage pulses are such as to cause the electroporation of DNA-containing material located therebetween.

In this embodiment the pulse generator 33 is configured to generate electroporation voltage pulses having a square waveform with a voltage of 125 V, which voltage corresponds to an electroporation field strength of 12.5 kV/cm, a duration of 500 μs, and an interval (relaxation time) of 20 ms. In preferred embodiments the voltage of the electroporation voltage pulses is such as to provide an electroporation field strength of from about 500 V/cm to about 12.5 kV/cm, preferably from about 2 kV/cm to about 12.5 kV/cm. In preferred embodiments the duration of the electroporation voltage pulses is from about 20 μs to about 1 ms. In preferred embodiments the interval of the electroporation voltage pulses is from about 2 ms to about 100 ms, preferably from about 5 ms to about 50 ms.

In other embodiments the pulse generator 33 could be configured to provide electroporation voltage pulses having a waveform other than square, such as a capacitive discharge which has an exponential decay. In preferred embodiments the capacitive discharge pulses would have a duration of from about 1 ms to about 10 ms, and preferably from about 2 ms to about 6 ms.

The DNA extraction system further comprises a voltage supply 35 which is connected to the electroseparation electrodes 27a, 27b and operable to apply an electroseparation voltage thereacross, which electroseparation voltage is such as to generate an electroseparation field which causes the electrophoretic separation of DNA from the electroporated DNA-containing material in the flow of sample into the parallel flow of separation medium, thereby providing for the separation of DNA from DNA-containing material in the sample.

In this embodiment the voltage supply 35 is configured to provide an electroseparation field strength of 370 V/cm. In preferred embodiments the electroseparation field strength if from about 60 V/cm to about 700 V/cm.

The DNA extraction system further comprises a control unit 37, in this embodiment a personal computer, for controlling the delivery units 29, 31, the pulse generator 33 and the voltage supply 35, in this embodiment from a LabView program (National Instruments, Austin, Texas, US). In this embodiment the delivery units 29, 31 are operated to provide flows of substantially the same rate. In another embodiment the separation medium delivery unit 31 could be configured to deliver separation medium at a higher flow rate than the sample delivery unit 29, such as to ensure that none of the sample flow enters the DNA outlet channel 15. It will be understood that a consequence of such flow rates is that a part of the separation medium flow, which entrains an amount of the extracted DNA, will pass into the sample waste channel 19 and be exhausted to waste.

Figure 3 illustrates a DNA extraction system in accordance with a second embodiment of the present invention.

The DNA extraction system of this embodiment is very similar to that of the above-described first embodiment, and thus, in order to avoid any unnecessary duplication of description, only the differences will be described in detail, with like parts being designated by like reference signs.

The DNA extraction system of this embodiment differs from that of the above-described first embodiment only in that the electrode unit 23 comprises a single pair of electrodes 45a, 45b which act as combined electroporation and electroseparation electrodes and to which the pulse generator 33 and the voltage supply 35 are both connected.

Operation is the same as for the above-described first embodiment, with the electroporation voltage pulses being applied over the background electroseparation voltage.

Figure 4 illustrates a DNA extraction system in accordance with a third embodiment of the present invention.

The DNA extraction system of this embodiment is very similar to that of the above-described first embodiment, and thus, in order to avoid any unnecessary duplication of description, only the differences will be described in detail, with like parts being designated by like reference signs.

The DNA extraction system of this embodiment differs from that of the above-described first embodiment only in that the electroporation electrodes 25a, 25b are located at the sample delivery channel 5. With this configuration, DNA-containing material in a sample is electroporated prior to reaching the main channel 3, whereby the electrophoretic effect of the electroporation voltage pulses on the DNA-containing material in the main channel 3 is obviated.

Operation is the same as for the above-described first embodiment, but with electroporation of DNA-containing material in the sample occurring in the sample delivery channel 5.

Finally, it will be understood that the present invention has been described in its preferred embodiments and can be modified in many different ways without departing from the scope of the invention as defined by the appended claims.

For example, the microfabricated DNA extraction device could be combined with other microfabricated DNA analysis devices, such as PCR, separation and sequencing devices, to provide a micromachined total analysis system (μ-TAS). Such a system would allow for the rapid analysis of very small amounts of complex samples, which samples would require no pre-treatment, as DNA extraction would be achieved by the DNA extraction device 1 of the present invention.

In another embodiment a plurality of the DNA extraction devices 1 could be connected in series in order to increase the DNA yield from a sample. In one embodiment the plurality of DNA extraction devices 1 could be provided in a single microchip.

CLAIMS

1. A DNA extraction microchip for extracting DNA from a sample including DNA-containing material, the microchip including:

a main channel through which first and second parallel flows are in use delivered, the first flow being of a sample including DNA-containing material and the second flow being of a separation medium; and

an electrode unit for applying a first, electroporation field to the first flow of sample including DNA-containing material, the electroporation field being such as to effect electroporation of DNA-containing material, and a second, electroseparation field across the main channel, the electroseparation field being such as to effect electroseparation of DNA from the electroporated DNA-containing material in the first flow into the second, parallel flow.

2. The microchip of claim 1, wherein the main channel has a length of from about 1.5 mm to about 12 mm.

3. The microchip of claim 1 or 2, wherein the main channel has a width of from about 100 μm to about 1.04 mm.

4. The microchip of claim 3, wherein the main channel has a width of from about 100 μm to about 240 μm.

5. The microchip of any of claims 1 to 4, wherein the main channel has a depth of from about 10 μm to about 100 μm.

6. The microchip of any of claims 1 to 5, further including:

a sample delivery channel fluidly connected to one, upstream end of the main channel through which the first flow of sample including DNA-containing material is in use delivered; and

a separation medium delivery channel fluidly connected to the one end of the main channel through which the second flow of separation medium is in use delivered.

7. The microchip of claim 6, wherein the sample delivery channel is connected to one side of the one end of the main channel such that the first flow is along the one side of the main channel, and the separation medium delivery channel is connected to the other side of the one end of the main channel such that the second flow is along the other side of the main channel.

8. The microchip of claim 6 or 7, wherein the sample delivery channel is inclined at an acute angle to the first and second flows.

9. The microchip of any of claims 6 to 8, wherein the separation medium delivery channel is inclined at an acute angle to the first and second flows.

10. The microchip of claim 8 or 9, wherein the acute angle is approximately 45 degrees.

11. The microchip of any of claims 1 to 10, further including:
a DNA outlet channel fluidly connected to the other, downstream end of the main channel through which the second flow of separation medium entraining extracted DNA is in use directed.

12. The microchip of claim 11, wherein the DNA outlet channel is connected to the other side of the other end of the main channel such that the second flow is directed therethrough.

13. The microchip of claim 11 or 12, wherein the DNA outlet channel is inclined at an acute angle to the first and second flows.

14. The microchip of claim 13, wherein the acute angle is approximately 45 degrees.

15. The microchip of any of claims 11 to 14, further including:
a sample waste channel fluidly connected to the other end of the main channel through which the first flow of sample, less extracted DNA, is directed.

16. The microchip of claim 15, wherein the sample waste channel is connected to the one side of the other end of the main channel such that the first flow is directed therethrough.

17. The microchip of claim 15 or 16, wherein the sample waste channel is inclined at an acute angle to the first and second flows.

18. The microchip of claim 17, wherein the acute angle is approximately 45 degrees.

19. The microchip of any of claims 1 to 18, wherein the electrode unit comprises first, electroporation electrodes and second, electroseparation electrodes disposed downstream of the electroporation electrodes.

20. The microchip of claim 19, wherein the electroseparation electrodes are disposed adjacent the main channel.

21. The microchip of claim 19, wherein the electroporation electrodes are disposed adjacent the main channel.

22. The microchip of claim 19 when appendant upon claim 6, wherein the electroporation electrodes are disposed adjacent the sample delivery channel.

23. The microchip of any of claims 19 to 22, wherein the electroseparation electrodes each have a length of from about 750 μm to about 10 mm.

24. The microchip of any of claims 19 to 23, wherein the electroporation electrodes each have a length of from about 150 μm to about 1 mm.

25. The microchip of any of claims 1 to 18, wherein the electrode unit comprises combined electroporation and electroseparation electrodes disposed adjacent the main channel.

26. A DNA extraction system for extracting DNA from a sample including DNA-containing material, the system comprising:
the microchip of any of claims 1 to 25.

27. The system of claim 26, further comprising:
a sample delivery unit for delivering a flow of a sample including a DNA-containing material to the microchip.

28. The system of claim 26 or 27, further comprising:
a separation medium delivery unit for delivering a flow of a separation medium to the microchip.

29. The system of claims 27 and 28, wherein the sample delivery unit and the separation medium delivery unit are operable to deliver flows having substantially the same flow rate.

30. The system of claims 27 and 28, wherein the sample delivery unit and the separation medium delivery unit are operable such that a flow rate of the delivered separation medium is higher than a flow rate of the delivered sample.

31. The system of any of claims 26 to 30, further comprising:
a pulse generator for applying electroporation voltage pulses across the electroporation electrodes.

32. The system of claim 31, wherein the pulse generator is configured to generate electroporation voltage pulses at a voltage corresponding to an electroporation field strength of from about 500 V/cm to about 12.5 kV/cm.

33. The system of claim 31 or 32, wherein the electroporation voltage pulses have a duration of from about 20 μs to about 1 ms.

34. The system of any of claims 31 to 33, wherein the electroporation voltage pulses have an interval of from about 2 ms to about 100 ms.

35. The system of any of claims 26 to 34, further comprising:
a voltage supply for applying an electroseparation voltage across the electroseparation electrodes.

36. The system of claim 35, wherein the voltage supply is configured to generate an electroseparation voltage at a voltage corresponding to an electroseparation field strength of from about 60 V/cm to about 700 V/cm.

37. A DNA extraction method for extracting DNA from a sample including DNA-containing material, the method comprising the steps of:
providing a DNA extraction microchip including a main channel;
delivering first and second parallel flows through the main channel, the first flow being of a sample including DNA-containing material and the second flow being of a separation medium;
applying an electroporation field to the first flow of sample including DNA-containing material such as to effect electroporation of DNA-containing material; and
applying an electroseparation field across the main channel such as to effect electroseparation of DNA from the electroporated DNA-containing material in the first flow into the second, parallel flow.

38. The method of claim 37, wherein the first flow is along one side of the main channel, and the second flow is along the other side of the main channel.

39. The method of claim 37 or 38, wherein the microchip includes a DNA outlet channel fluidly connected to one, downstream end of the main channel, and further comprising the step of:
directing the second flow of separation medium entraining extracted DNA through the DNA outlet channel.

40. The method of claims 38 and 39, wherein the DNA outlet channel is connected to the other side of the one end of the main channel such that the second flow is directed therethrough.

41. The method of any of claims 37 to 40, wherein the electroseparation field is applied across electroseparation electrodes disposed adjacent the main channel.

42. The method of any of claims 37 to 41, wherein the electroporation field is applied across electroporation electrodes disposed adjacent the main channel.

43. The method of any of claims 37 to 41, wherein the electroporation field is applied across electroporation electrodes upstream of the main channel.

44. The method of any of claims 37 to 40, wherein the electroporation field and the electroseparation field are applied across combined electroporation and electroseparation electrodes disposed adjacent the main channel.

45. The method of any of claims 42 to 44, wherein the electroporation field comprises a pulsed field of electroporation voltage pulses.

46. The method of claim 45, wherein the electroporation voltage pulses are at a voltage corresponding to an electroporation field strength of from about 500 V/cm to about 12.5 kV/cm.

47. The method of claim 45 or 46, wherein the electroporation voltage pulses have a duration of from about 20 μs to about 1 ms.

48. The method of any of claims 45 to 47, wherein the electroporation voltage pulses have an interval of from about 2 ms to about 100 ms.

49. The method of any of claims 41 to 48, wherein the electroseparation field comprises a substantially continuous field of an electroseparation voltage across the electroseparation electrodes.

50. The method of claim 49, wherein the electroseparation voltage is at a voltage corresponding to an electroseparation field strength of from about 60 V/cm to about 700 V/cm.

51. The method of any of claims 37 to 50, wherein the first and second flows have substantially the same flow rates.

52. The method of any of claims 37 to 50, wherein a flow rate of the second flow is higher than a flow rate of the first flow.

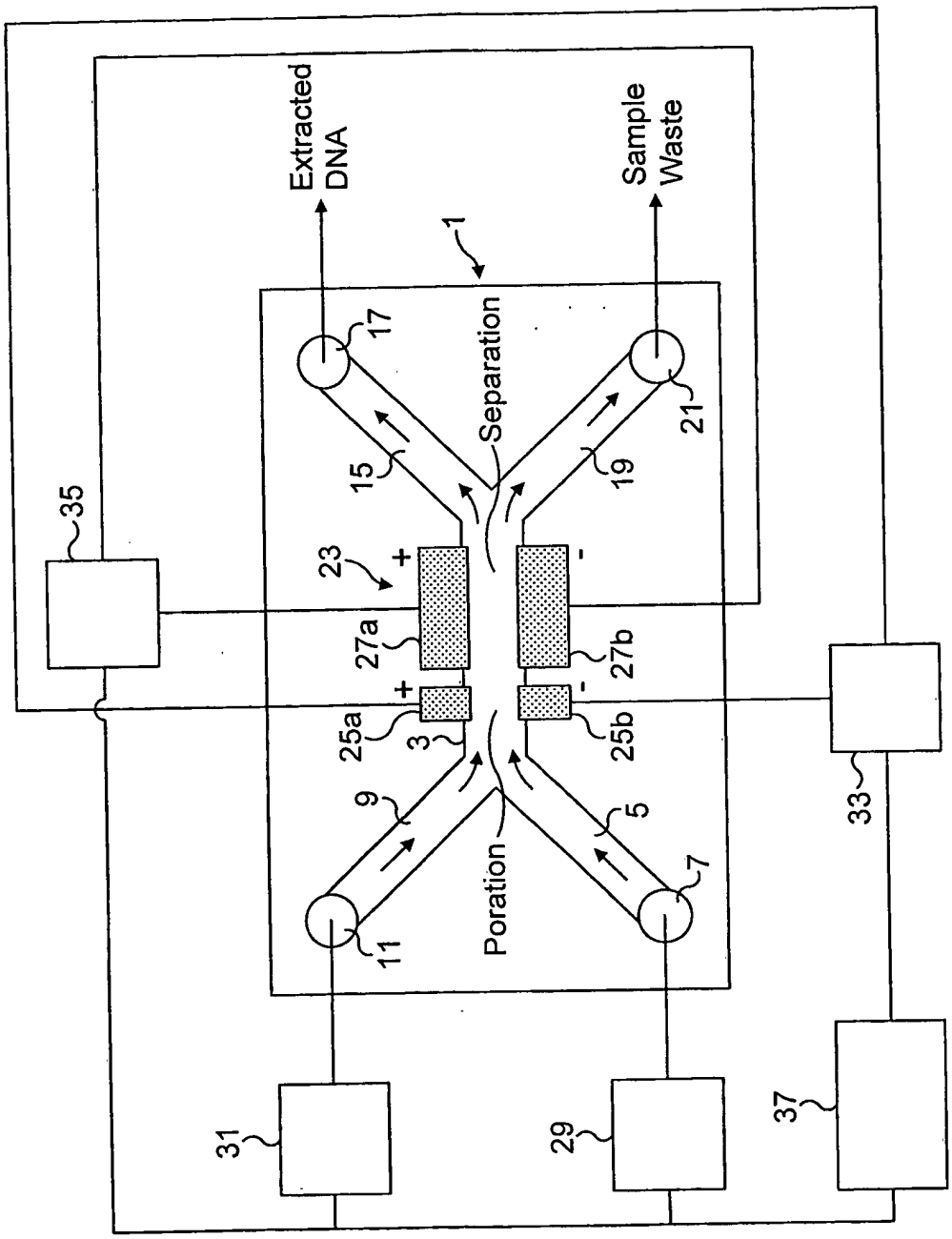
Extracted DNA
Sample Waste
1
17
Separation
21
15
19
35
23
+
-
27a
27b
+
-
25a
3
25b
9
5
33
Poration
7
11
31
29
37

FIG. 1

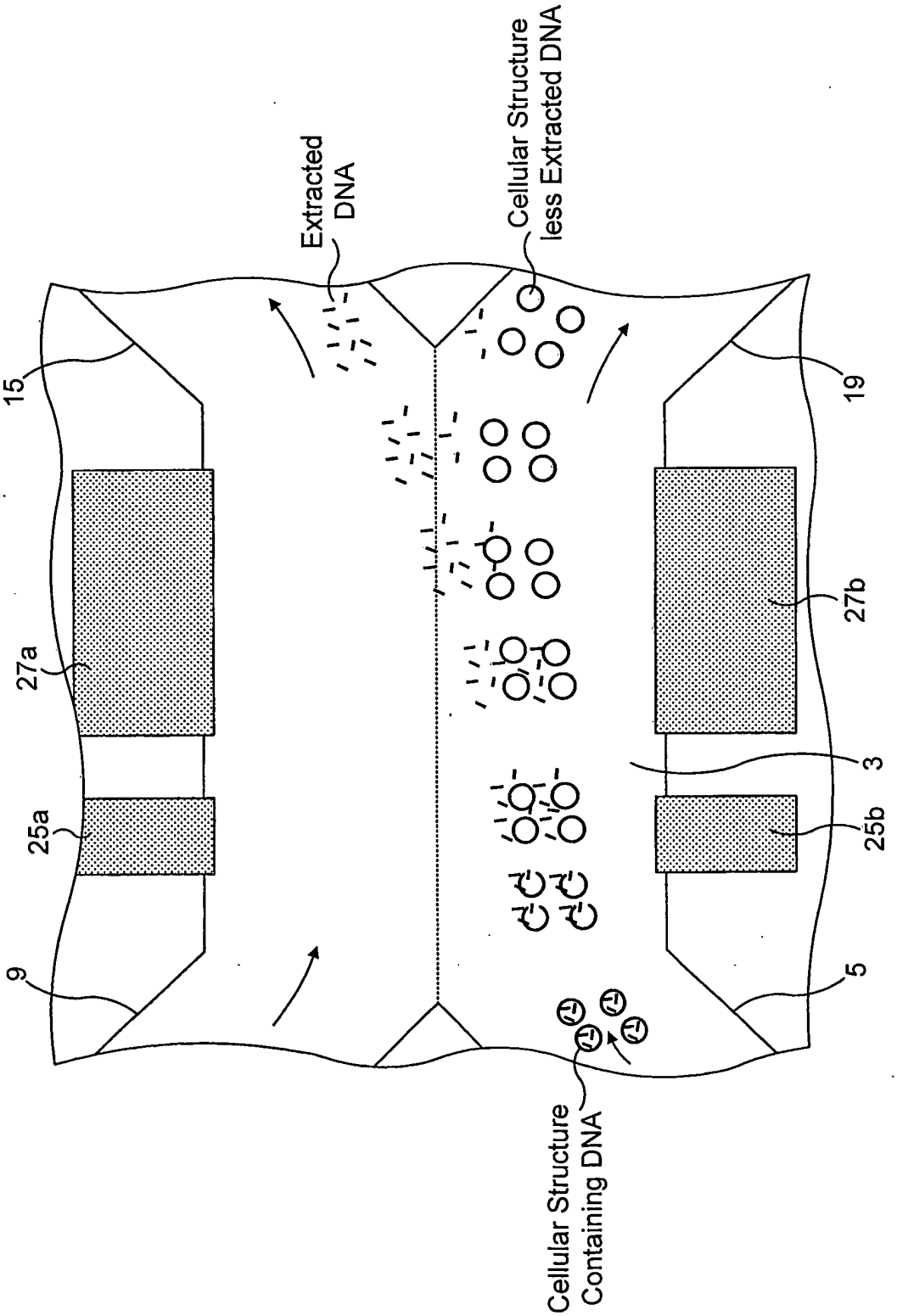
Extracted DNA
Cellular Structure less Extracted DNA
15
19
27a
27b
25a
25b
3
9
5
Cellular Structure Containing DNA

FIG. 2

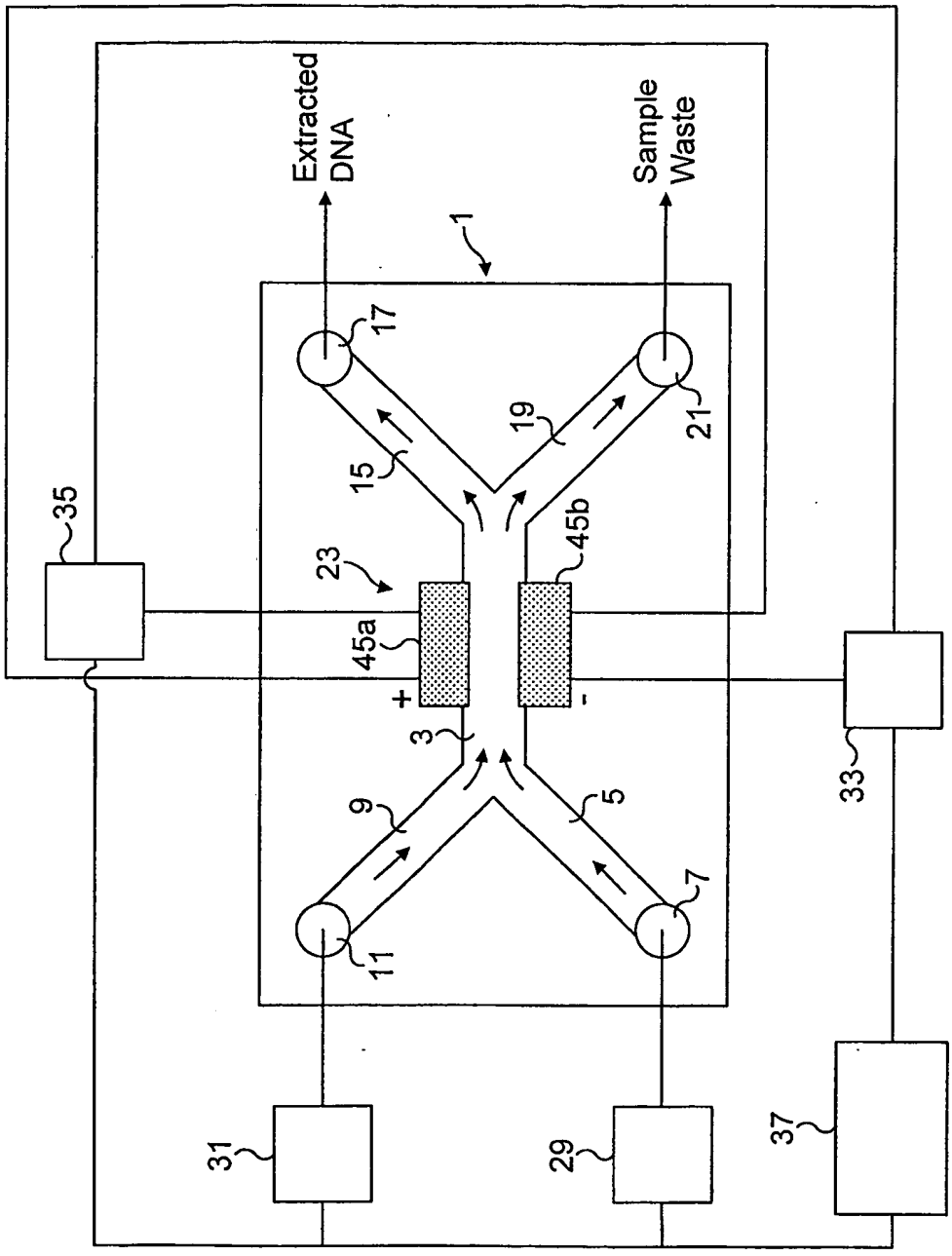
Extracted DNA
Sample Waste
1
17
19
21
15
35
23
45b
45a
+
-
3
9
5
7
11
33
31
29
37

FIG. 3

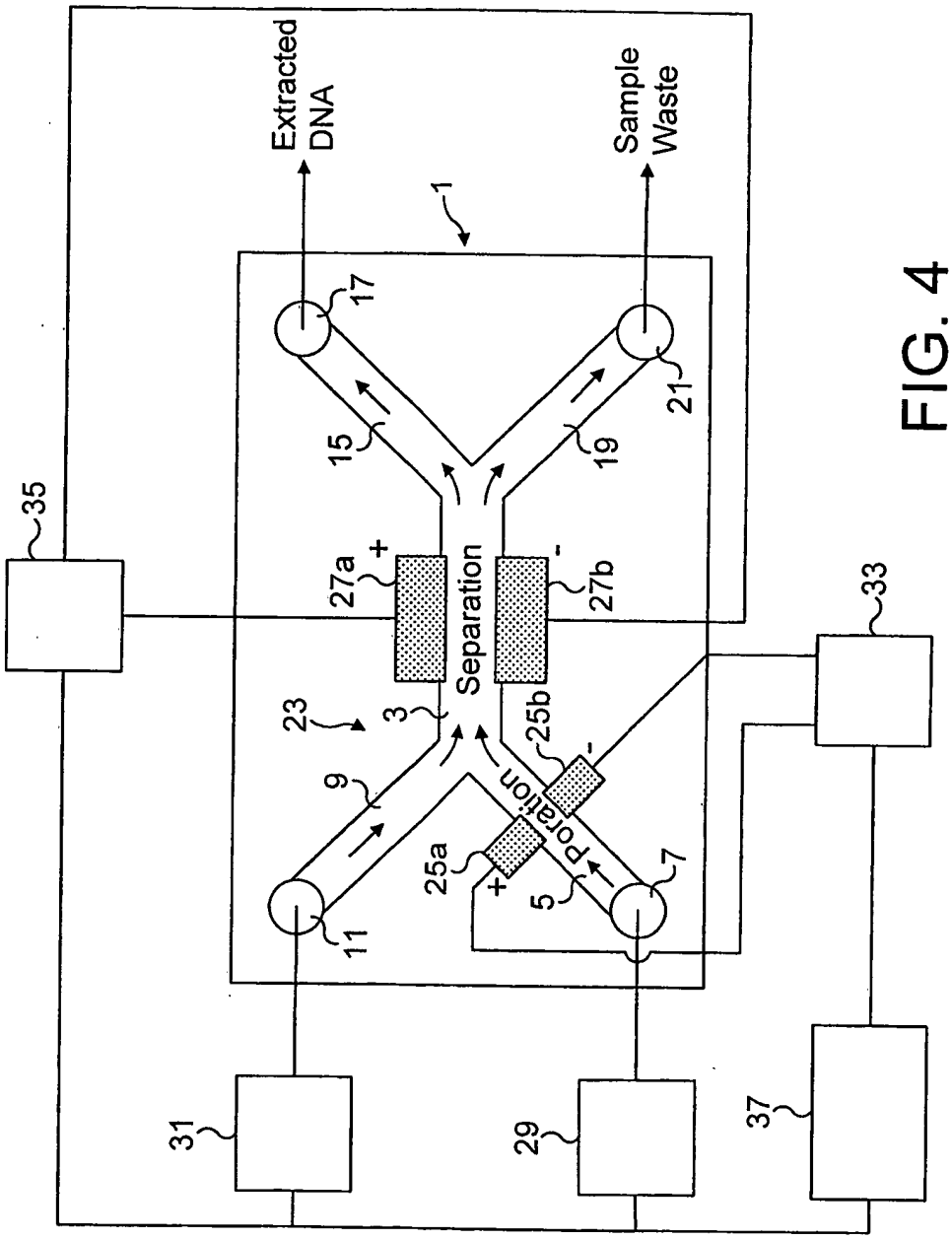
Extracted DNA
Sample Waste
1
17
21
15
19
35
27a
+
Separation
-
27b
33
23
3
25b
9
-
Pollution
25a
+
5
7
11
31
29
37

FIG. 4

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 03/03328

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/10 C12M1/33 G01N30/00 G01N27/447 B01J19/00
C12M1/42

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C12M G01N B01J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, INSPEC, BIOSIS, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6 136 171 A (CALDWELL KARIN D ET AL) 24 October 2000 (2000-10-24) the whole document ---	1-52
A	US 5 180 480 A (MANZ ANDREAS) 19 January 1993 (1993-01-19) the whole document ---	1-52
A	WO 99 38612 A (NANOGEN INC) 5 August 1999 (1999-08-05) p. 5, line 30 - line 32 p. 7, line 4 - line 10 page 6, line 16 - line 21 ---	1-52
A	WO 88 02777 A (ELECTROPORE INC) 21 April 1988 (1988-04-21) the whole document ---	

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 November 2003	05/12/2003

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 03/03328

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4 894 146 A (GIDDINGS JOHN C) 16 January 1990 (1990-01-16) the whole document	

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internati[redacted]Application No
PCT/GB 03/03328

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